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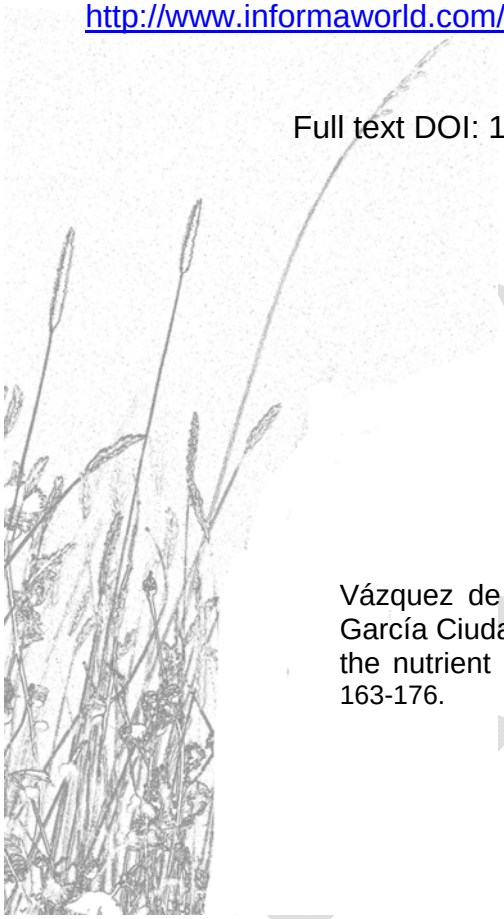

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Influence of Fungal Endophyte Infection on the Nutrient Content of Tall Fescue

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ABSTRACT

Fungal endophytes infect several grass species. The fungus can alter the growth, physiological and morphological characteristics of the infected plant. A greenhouse experiment was designed to determine the effect of the fungal endophyte *Neotyphodium coenophialum* on the nutrient content of a selected ecotype of tall fescue (*Festuca arundinacea*), when growing at two nutrient supply levels. Stem dry matter, averaged over all harvests and nutrient supply treatments, was higher in non-infected (E-) than in endophyte infected (E+) plants. We found a significant interaction between endophyte infection and nutrient supply level and/or harvest date on the N, P, Ca and Mg plant tissue concentrations. Only for Ca leaf concentrations the effect of infection status was not influenced by harvest date or nutrient supply treatment and Ca concentration was higher in E- than in E+ plants. Differences between E+ and E- plants in P concentration were significant at the beginning of the experiment, and for N and Mg were observed at the end of the experiment (16-18 weeks). Endophyte infected plants had higher stem N and leaf Mg concentrations in the high nutrient supply but significantly lower concentrations at the low nutrient supply treatment. Phosphorus

concentration in leaf and aboveground plant tissue was significantly higher in E- than in E+ plants at the high nutrient fertility treatment at week 12, but in the low fertility treatment differences were not significant. Differences in K, Fe, Mn, Zn and Cu concentration between infected and non-infected plants were not statistically significant. Our results suggest that endophyte infection of tall fescue appears to increase the content of nutrients related to protein synthesis processes (N and Mg).

INTRODUCTION

The association between clavicipitaceous fungal endophytes of the genera *Neotyphodium* and *Epichloë* and grass species has been lately focused in many researches. Most endophytic fungus-grass associations have been described as mutualistic (Bacon et al., 1986; Clay, 1988). The fungus receives nutrients, protection and means of dispersion from the plant. The fungus confers to the host plant resistance to herbivores by means of toxin production. Several endophyte infected grasses have been reported to contain alkaloids such as peramine (Tapper et al., 1989), which deters insect feeding, or ergovaline which is toxic for cattle (Lyons et al., 1986; Siegel et al., 1990).

Infection by endophytes of the genera *Neotyphodium* are common in several pasture grasses: *Festuca arundinacea*, *Lolium perenne*, and *Festuca pratensis* (Siegel et al., 1987; Clay, 1990; Zabalgogeazcoa et al., 1997). Tall fescue (*Festuca arundinacea*), a widely grown forage grass, is often infected by *Neotyphodium coenophialum* (Morgan and Gams) (Glenn et al., 1996) endophytes. Endophytes can alter the growth, morphological and physiological characteristics of the host plant, thereby influencing the persistence and survival rate of infected plants (Clay, 1994). It has been reported that under certain conditions infected tall

fescue plants can produce more dry matter (Clay, 1987; Arechavaleta et al., 1989; De Battista et al., 1990; Bouton et al., 1993) and are more drought tolerant (Read and Camp, 1986; West et al., 1988; Arechavaleta et al., 1989) than non-infected plants. Other beneficial effects of endophyte infection observed in the host plants are resistance to nematodes and several fungal diseases (West et al., 1988 ; Schardl, 1996).

There is scarce information about the influence of endophyte infection on the nutrient content of the host plant. Lyons et al. (1990) have found out that the fungus can affect N metabolism in both leaf sheath and leaf blade. They reported that the total free nitrogen of leaves of a genotype of tall fescue was decreased by endophyte infection at any rate of N fertilization. Recently, Malinowsky et al. (1998) reported the influence of phosphorus level on the growth and nutrient concentration of endophyte infected tall fescue plants. Their results suggest that endophyte infection affects uptake of phosphorus and may benefit tall fescue grown on P-deficient soils. The aim of this study was to determine the effect of the infection by *Neotyphodium coenophialum* on the nutrient content (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) of the "Her-1" ecotype of tall fescue.

MATERIAL AND METHODS

Plant Material

Festuca arundinacea plants of the line "Her-1" were used in this study. Endophyte free and endophyte infected seeds of this line were obtained as follows: seeds from a single infected plant were divided in two groups, one group was treated with triadimenol fungicide (Bayfidan 25 EC, Bayer) 4.8 g kg^{-1} of seed (Williams et al., 1984), and the other group was not treated. Fungicide treated and non-treated seeds were planted, and these plants did

produce the endophyte-free (E-) and endophyte-infected (E+) seed which was used in this study. The endophyte infection status of mother plants and progeny seeds was verified by aniline blue staining (Bacon et al., 1977). The infection rate in the E+ seeds was 98% and in the E- seeds of 0%.

Experimental Design

Endophyte infected (E+) and non-infected (E-) seeds were planted in flats containing peat. The experiment was carried out in a greenhouse during the 1997 spring-summer time. Ten day old seedlings of E+ and E- plants were transplanted into 2.8 liter pots containing perlite substrate. Each pot contained five individual plants. The experiment was designed with two nutrient supply levels, high fertility (HF) and low fertility (LF), and four harvest dates. Each block, infection status × nutrient supply level × harvest date, was replicated three times. Nutrient fertility treatments were a 2:3 dilution (HF) and a 1:12 dilution (LF) of the solution described by Hoagland and Arnond (Hewitt, 1966). The HF solution contained 1.7 g l^{-1} NO_3K; 3.93 g l^{-1} $Ca(NO_3)_2 \cdot 4H_2O$; 1.63 g l^{-1} $MgSO_4 \cdot 7H_2O$; 453 mg l^{-1} KH_2PO_4; 9.55 mg l^{-1} H_3BO_3; 6.03 mg l^{-1} $MnCl_2 \cdot 4H_2O$; 266 µg l^{-1} $CuSO_4 \cdot 5H_2O$; 733 µg l^{-1} $ZnSO_4 \cdot 7H_2O$; 300 µg l^{-1} H_2MoO_4, and 5 mg l^{-1} $(C_4O_6H_4)_3Fe_2$. The LF solution contained: 210 mg l^{-1} NO_3K; 487 mg l^{-1} $Ca(NO_3)_2 \cdot 4H_2O$; 202 mgl^{-1} $MgSO_4 \cdot 7H_2O$; 56 mg l^{-1} KH_2PO_4; 3.81 mg l^{-1} H_3BO_3; 2.41 mg l^{-1} $MnCl_2 \cdot 4H_2O$; 106 µg l^{-1} $CuSO_4 \cdot 5H_2O$; 293 µg l^{-1} $ZnSO_4 \cdot 7H_2O$; 120 µg l^{-1} H_2MoO_4, and 1 mg l^{-1} $(C_4O_6H_4)_3Fe_2$. The pH was adjusted to the 5-6 range by addition of NaOH. To each pot, 0.2 l of the appropriate nutrient solution were applied once a week. Plants were watered as needed between nutrient applications.

Twelve pots (2 infection levels × 2 nutrient treatments × 3 replicates) were harvested at 12, 14, 16 and 18 weeks after emergence. At the last harvest plants were at the stem extension stage. At each harvest, plants were divided into leaf blades and pseudostems

(sheaths). Plant samples were dried at 60 ºC for 24 hours and ground in a Ultracentrifugal Retsch ZM1 mill to a sieve size of 0.5 mm. Nitrogen (N) concentration in plant tissue was determined by Kjeldahl digestion. Plant tissue mineral contents were determined by ashing plant tissue in a muffle furnace (Duque Macias, 1970). Phosphorus (P) concentrations were then measured colorimetrically as molybdovanadate-phosphoric acid. Potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were determined by atomic absorption spectrophotometry.

Statistical Analysis

A three-way analysis of variance with endophyte infection, nutrient supply treatment and harvest date as main factors was performed for dry matter and macronutrient concentration data. Previously a fourth factor - replication - was included in the anova. As in all plant measurements the replication factor was not statistically significant, it was excluded from subsequent analysis. A two-way ANOVA with endophyte infection and nutrient supply treatment as main factors was used to determine significant differences in the micronutrient concentrations at the last harvest (week 18). Least significant differences (LSD) test was used to determine whether differences between means were statistically significant.

RESULTS AND DISCUSSION

Biomass Production

Analysis of variance did not reveal any significant effect of endophyte infection on the biomass of leaves ($P>0.05$) but the interaction infection × fertilization level × harvest (I × F × H) was statistically significant ($P<0.001$, Table 1). In the high nutrient supply treatment (HF)

dry matter of leaves was higher in non-infected plants (E-) than in endophyte infected plants (E+) at week 14 (Figure 1). Endophyte infection had a significant effect on the biomass production of stems ($P<0.05$). Stem dry matter, averaged over all harvests and nutrient supply treatments, was higher in E- plants as compared to E+ plants (Table 2). In the high nutrient supply treatment non-infected plants had higher total aboveground dry matter than infected plants at week 14 ($I \times F \times H$ interaction, Table 1; Figure 1).

We did not find great differences in the leaf or total aboveground dry matter production between infected and non-infected plants, however a significant effect of endophyte infection on the stem dry matter was found. These results agree in part with those of Belesky et al. (1989a) who found that leaf blade yield in tall fescue was not significantly influenced by endophyte status, however pseudostem yield was affected by the interaction between endophyte status and genotype. Our results showed that in all cases where differences occur, E- plants had higher dry matter than E+ plants. This could suggest a depletion of nutrients available for metabolic processes in endophyte infected plants due to nutrient uptake by the fungus (Cheplick et al. 1989). This implies an extra nutrient cost to the host plant.

The results of other studies have shown that E+ plants of *Festuca arundinacea* can produce significantly more aboveground biomass per unit time than E- plants when growing in greenhouse (Clay, 1987; De Battista et al., 1990) or field conditions (Read and Camp, 1986). However, Malinowsky et al. (1998) found that with increased P availability in the soil, shoot and root dry matter in E+ plants of tall fescue were significantly less when compared to E- plants. Thus, they suggested that endophyte infected tall fescue is not responsive to high soil P (Malinowski et al., 1998). It seems that an interaction between endophyte infection and abiotic factors exists. The advantage of endophyte infection has been found to diminish at lower nutrient levels (Arechavaleta et al., 1989; Cheplick et al., 1989), or to be apparent only

where drought stress was evident (West et al., 1988; Arechavaleta et al., 1989). Cheplick et al. (1989) reported that, as compared to non-infected plants, infected seedlings of tall fescue had greater biomass at the high nutrient level but a significantly lower biomass production at the low nutrient level which presumably reflects the balance between the advantage of infection and the cost to the host of supporting the endophyte. The reason why we did not find an increased yield of infected plants at the high nutrient level could be due to differences in the experimental conditions. Although the rate of nutrients in our experiment (calculated on plant basis) was similar to that of Cheplick et al. (1989), their experiment was carried out in more favorable conditions: environmental growth chamber, a richer soil mixture was used, and one plant per pot was planted. It could also be suggested that differences between infected and non-infected plants could not occur only where nutrients are limiting as suggested by Cheplick et al. (1989), or that the magnitude of the endophyte impact upon the tall fescue productivity depends on the plant genotype, as indicated by Sleper et al. (1980), and consequently the dry matter production of the "Her-1" ecotype is not increased by endophyte infection at the nutrient level of the experiment.

Nutrient Content

There were no significant differences between infected and non-infected plants in N concentration of leaves, not even within nutrient supply treatments or harvest dates (Table 1). In the high nutrient supply treatment, N concentration in stems was higher in endophyte infected than in non-infected plants ($P<0.01$, $I \times F$ interaction, Table 1; Figure 2). The N concentration in total aboveground biomass was higher ($P<0.01$, $I \times F \times H$ interaction, Table 1) in E+ than in E- plants when growing in the high nutrient supply treatment at week 18, but in the LF treatment N concentration in E+ plants was lower ($P<0.01$) than in E- plants at week 16 (Table 1; Figure 2). These results would suggest, that differences in shoot N concentration

between E+ and E- plants depend to some extent on the plant development stage. Our results showed that an interaction between endophyte infection and nutrient treatment also exits.

The differences in stem N concentration are not related to a dilution effect since differences in stem dry matter production were not affected by the I × F interaction. Endophyte-infected plants contain alkaloids produced by the fungus (Lyons et al. 1986). The increase in stem N concentration in E+ plants with increasing nutrient supply could be interpreted as the N involvement in secondary metabolites produced by the fungus (i.e. ergot alkaloids, peramine). Ergot alkaloid production by infected tall fescue is known to be enhanced by the addition of nitrogen fertilizer (Lyons et al., 1986). The higher ergot alkaloid concentration in leaf sheaths than in blades of tall fescue (Lyons et al., 1986; Belesky et al. 1989b) could explain why the leaf N concentration was not affected by the plant infection status. Lyons et al. (1990) have found that the total free nitrogen of leaves of the K31 tall fescue genotype was decreased by endophyte infection even though the plants were fertilized with a high rate of N. An interpretation of this result is that endophyte infected grasses are utilizing this free pool of N for the synthesis of fungus-specific compounds (Lyons et al., 1990). We found that the mycelium from a potato dextrose agar (PDA) culture of a related fungal endophyte, *Epichloë festucae*, contained a 3.3% of N (Kjeldahl). This could suggest an increase in N content of a plant by factors other than the production of secondary metabolites by the fungus. Such an increase could be related to the concentration of fungus within the plant, which is highest in the base of leaf sheath (Hinton and Bacon, 1985). Our results also indicate that dry matter production per unit of absorbed nitrogen is higher in E- than in E+ plants, which means a lower nitrogen productivity in endophyte infected plants.

Phosphorus concentrations in leaves and aboveground tissue were affected by the I × F × H interaction ($P<0.01$, Table 1). In the high fertility treatment, E- plants had a higher P concentration than E+ plants at week 12 (Figure 2). The effect of endophyte status on the P

concentration had opposite sign to the N concentration at the high nutrient fertility treatment. These results agreed in part with those of Malinowski et al. (1998) who found that P concentration was higher in E- than in E+ plants of tall fescue in the high soil-P level, but the opposite effect was observed at the low soil-P level.

Potassium concentration in leaves or stems did not differ between infected or non-infected plants, as indicated by ANOVA (Table 1; Figure 2). Wilkinson and Mayland (1997) compared mineral concentration of different tall fescue cultivars (E+ and E-) and found that K concentration was not different.

Mean Ca concentration, averaged over all harvests and nutrient treatments (Table 2), was lower in leaves of infected plants than in non-infected plants ($P<0.05$, Table 1). Differences in stem Ca concentration between E+ and E- plants were not statistically significant (Table 1). Calcium concentration in aboveground tissue was significantly higher in E- than in E+ plants at weeks 12 and 14 ($P<0.05$, $I \times H$ interaction, Table 1; Figure 3). Malinowski et al (1998) reported higher Ca concentration in E+ plants when grown at low soil-P level. Differences in Ca concentration between E+ and E- plants could suggest an influence of the endophyte infection status upon the fiber content since Ca is an important structural element of the cell wall. Thus, according to our results infected plants would have lower fiber content than non-infected plants. It has been reported that neutral detergent fiber (NDF) and acid detergent fiber (ADF) were not affected in tall fescue due the presence of fungus (Bush and Burrus, 1988). However we found that infected plants had lower ADF and NDF than non-infected plants (unpublished data).

The analysis of variance showed a significant effect of the interaction $I \times F \times H$ ($P<0.05$, Table 1) on the Mg concentration of leaves. In the low fertilized treatment the leaf Mg concentration was higher in E- than in E+ plants at week 16 (Figure 3). However when increased nutrient supply, the leaf Mg concentrations of E+ plants were higher than E- plants

(at week 18). Differences in stem Mg concentrations between infected and non infected plants were not statistically significant (Table 2). In the HF treatment, E+ plants had higher Mg concentration in aboveground tissue than E- plants at week 18. These results could suggest an increase in the chlorophyll content due to endophyte infection, although only a small percentage of the Mg would be attributable to the chlorophyll molecule. Magnesium has an essential function in protein synthesis process. Then, our results could suggest that the alteration of N metabolism due to the presence of the endophyte, as indicated by Lyons et al. (1990), could lead to an increase in Mg concentration which is related to protein synthesis process.

Table 2 shows mean micronutrient concentrations at week 18 (stem extension stage). The analysis of variance with infection and fertilization level as main factors did not show any significant difference ($P>0.05$, Table 3) between infected and non-infected plants in Fe, Mn, Zn and Cu concentrations. Nevertheless, aboveground Zn concentration was higher in E+ plants than in E- (Table 2) at $P=0.098$.

CONCLUSIONS

This study shows that endophyte infection have an influence on the nutrient content of tall fescue. Fungal endophyte infection increased the N and Mg contents and diminished the P and Ca concentrations of tall fescue. Our results suggest that endophyte infection of tall fescue appears to increase the content of nutrients directly related to protein synthesis processes (N, Mg). However, dry matter production was not increased due to the presence of the fungus indicating a lower nitrogen productivity in endophyte infected plants. These are important steps that can affect competitive abilities of infected plants and thus persistence and

dominance in communities. Further research experiments involving other infected grass species are required before the effect of fungus on the host nutrition can be determined.

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TABLE 1. Analysis of variance (P values) for dry matter production and N, P, K, Ca and Mg concentrations of leaves, stems and total aboveground tissue (Aboveg).

	Infection (I)	Fertilization (F)	Harvest (H)	I × F	I × H	I × F × H
Dry matter						
Leaf	0.975	0.000***	0.000***	0.182	0.603	0.000***
Stem	0.050*	0.000***	0.000***	0.109	0.564	0.000***
Aboveg	0.328	0.000***	0.000***	0.121	0.921	0.000***
N						
Leaf	0.130	0.000***	0.000***	0.267	0.192	0.000***
Stem	0.389	0.000***	0.000***	0.008**	0.012*	0.050*
Aboveg	0.781	0.000***	0.000***	0.031*	0.237	0.010**
P						
Leaf	0.890	0.000***	0.000***	0.889	0.246	0.008**
Stem	0.185	0.000***	0.000***	0.395	0.454	0.126
Aboveg	0.643	0.000***	0.000***	0.688	0.199	0.002**
K						
Leaf	0.411	0.000***	0.000***	0.411	0.526	0.508
Stem	0.750	0.000***	0.000***	0.873	0.885	0.000***
Aboveg	0.669	0.000***	0.000***	0.514	0.515	0.011**
Ca						
Leaf	0.050*	0.004**	0.000***	0.132	0.070	0.167
Stem	0.484	0.000***	0.000***	0.102	0.140	0.118
Aboveg	0.105	0.000***	0.000***	0.066	0.030*	0.450
Mg						
Leaf	0.529	0.000***	0.002**	0.084	0.322	0.000***
Stem	0.448	0.000***	0.000***	0.527	0.381	0.470
Aboveg	0.690	0.000***	0.144	0.102	0.210	0.000***

*** P<0.001 ; ** P<0.01 ; * P<0.05

TABLE 2. Mean values across nutrient fertility treatments and harvests for dry matter production (n = 24), macronutrient (n = 24) in E+ and E- plants. Micronutrient concentration values are means across fertility treatments at the last harvest (n = 6). LSD values at P<0.05 between E+ and E- plant parts.

	Leaf			Stem		
	E+	E-	LSD	E+	E-	LSD
Dry matter (g)	9.64	9.65	0.46	4.73	5.12	0.38
N (g kg^{-1})	23.02	23.42	0.51	16.67	16.35	0.75
P (g kg^{-1})	1.25	1.26	0.079	1.85	1.76	0.13
K (g kg^{-1})	27.04	27.52	1.17	24.47	24.31	1.05
Ca (g kg^{-1})	5.62	5.97	0.34	2.93	3.00	0.19
Mg (g kg^{-1})	2.52	2.48	0.13	1.89	1.94	0.13
Fe (mg kg^{-1})	41.66	42.5	10.87	41.25	44.58	17.6
Mn (mg kg^{-1})	49.58	47.5	6.45	39.58	39.58	4.73
Zn (mg kg^{-1})	22.08	19.06	6.19	55.66	42.91	19.9
Cu (mg kg^{-1})	2.50	2.91	1.76	2.50	2.75	0.76

TABLE 3 - Analysis of variance (P values) for Fe, Mn, Zn and Cu concentrations of leaves, stems and total aboveground tissue (Aboveg).

	Infection (I)	Fertilization (F)	I × F
Fe			
Leaf	0.799	0.030*	0.613
Stem	0.363	0.002**	0.815
Aboveg	0.675	0.000***	0.918
Mn			
Leaf	0.476	0.000***	0.882
Stem	1.000	0.090	0.162
Aboveg	0.326	0.000***	0.624
Zn			
Leaf	0.098	0.098	0.445
Stem	0.201	0.136	0.749
Aboveg.	0.098	0.098	0.445
Cu			
Leaf	0.346	0.346	0.346
Stem	0.346	0.346	0.346
Aboveg	0.346	0.346	0.346

*** P<0.001 ; ** P<0.01 ; * P<0.05

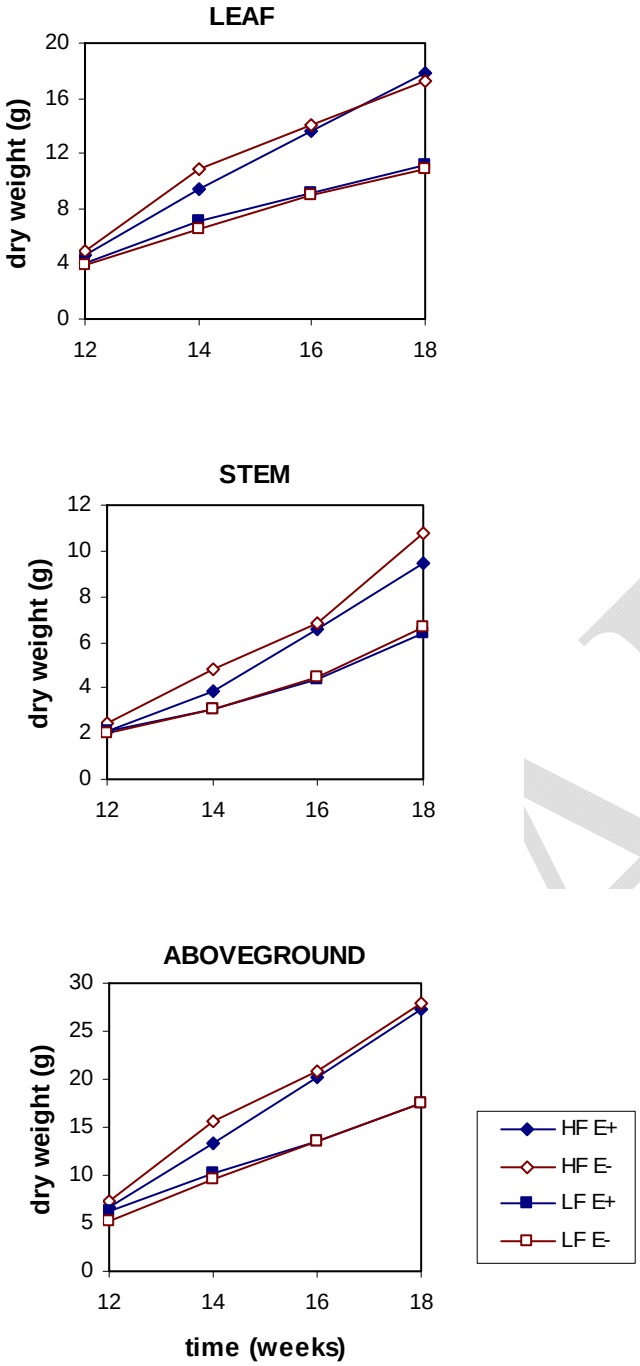

FIGURE 1. Biomass of leaves, stems, and total aboveground tissue (g dry matter pot^{-1}) of endophyte infected (E+) and non-infected (E-) plants in the high (HF) and low (LF) nutrient supply treatments (n=3).

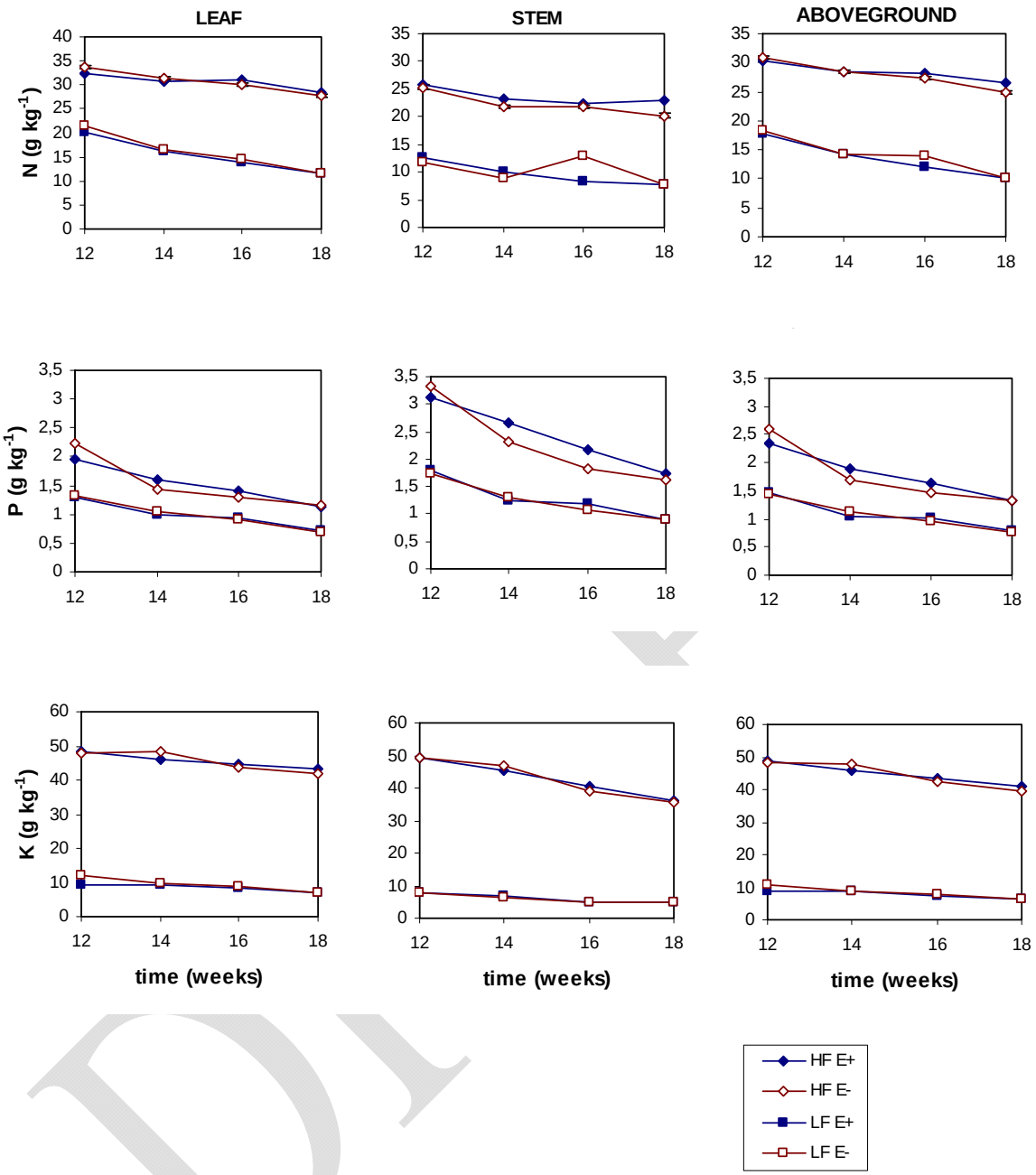

FIGURE 2. Concentrations of N, P and K (g kg^{-1}) in leaves, stems and total aboveground tissue of endophyte infected (E+) and non-infected (E-) plants in the high (HF) and low (LF) nutrient supply treatments (n=3).

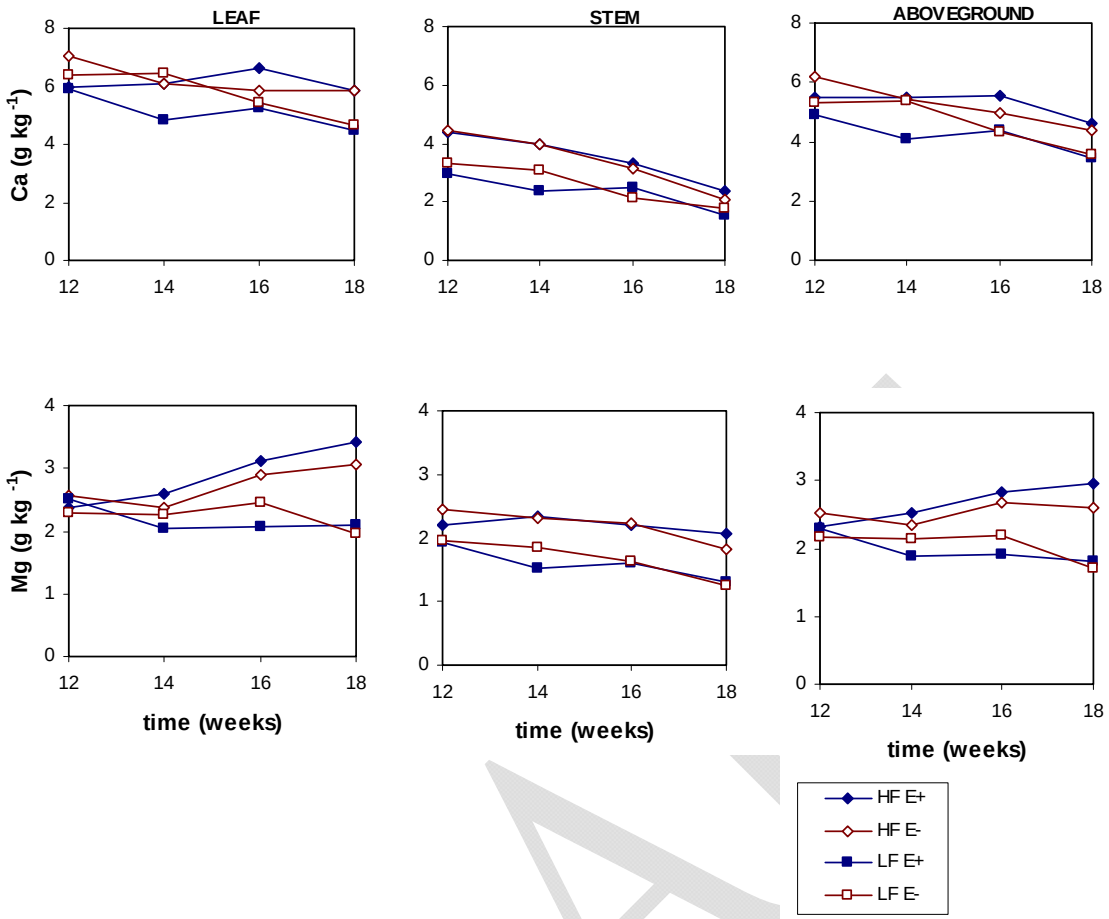

FIGURE 3. Concentrations of Ca and Mg (g kg^{-1}) in leaves, stems and total aboveground tissue of endophyte infected (E+) and non-infected (E-) plants in the high (HF) and low (LF) nutrient supply treatments (n=3).